Virus-like particles: a new family of delivery systems

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The development of antiviral vaccines has almost exclusively been based on live attenuated vaccines up until now. However, the efficacy of HBsAg particles as an antiHBV vaccine has clearly demonstrated that protective antiviral immunity can be achieved by other strategies. Virus-like particles formed by structural proteins were proven to be highly immunogenic and capable of inducing protective immunity against various viral infections in preclinical studies. Clinical trials using virus-like particles confirmed their safety and immunogenicity. Moreover, chimeric virus-like particles carrying foreign peptidic sequences were shown to elicit potent B- and T-cell responses. Virus-like particles formed by a fusion protein between the HBsAg and the circumsporozoite surface protein are safe and immunogenic in volunteers and induce a partial protection against natural *Plasmodium falciparum* infection.

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Most antiviral vaccines are based on attenuated live viruses. Purified proteins and killed viruses are usually inefficient to activate cellular antiviral immunity. However, the efficacy of the antihepatitis B vaccine, based on noninfectious virus-like particles (VLPs) has demonstrated that new, alternative strategies could be as efficient as attenuated live vaccines.

Structural proteins derived from a variety of viruses have indeed the ability to spontaneously assemble into particles, called virus-like particles that closely resemble virions. VLPs consist of highly repetitive and ordered structures that can trigger potent humoral responses and extremely powerful cellular responses. The fact that VLPs can induce protective antiviral immune responses without having the infectious and replicative capacities of the related virus represents a major advantage for the design of efficient and safe vaccines. In this review, we discuss recent developments of this strategy for the induction of therapeutic immunity against papilloma-induced cervical cancers, as well as the use of VLPs as new delivery vectors.

Papilloma VLP as vaccine against cervical cancers

Infection by certain human papillomavirus (HPV) is regarded as the major risk-factor in the development of cervical cancers, one of the most common cancers of women worldwide [1]. The development of an effective antiHPV vaccine based on attenuated virus has been hampered by the inability to efficiently propagate this virus *in vitro*. Conversely, the major capsid L1 PV protein produced in insect cells *via* a baculovirus vector is expressed at high levels and self assembles into structures that closely resemble HPV [1,2]. Furthermore, systemic immunization with VLPs based on L1 or L1/L2 VLPs was shown to protect against experimental papillomavirus infections in various animal models [1,3,4].

It is now well established that CD8+ T-cells play a central role in the eradication of infected cells and tumors. While high titer of neutralizing antibodies induced by PV-VLPs appeared as sufficient to prevent PV infection, therapeutic vaccine against PV-induced tumors would require induction of cytotoxic T-lymphocyte (CTL) responses to tumor epitopes. The E6 and E7 PV oncoproteins are expressed at all

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stages of tumor progression, from HPV infection to carcinoma development and are potential targets for activated T-cells for immunotherapy. In an effort to generate protective response against established tumors, PV-VLPs particles carrying the E7 oncoprotein were produced [5]. Chimeric L1 E7 protein between truncated L1 protein and the 60 amino-terminal acids of E7 remained able to form VLPs. Moreover, they also retained the capacity to induce neutralizing antibodies and also induced an E7-specific CTL response. Immunization with these chimeric VLPs prevented the outgrowth of E7-expressing tumor cells [1,5,6].

Several clinical trials were conducted using HPV-VLPs. Two recently published studies have established the safety and immunogenicity of these particles in volunteers. In a Phase I study, HPV Type 11 L1 VLPs were injected to 65 healthy adults without serologic evidence of previous HPV infection. The particles were well-tolerated and induced high levels of neutralizing antibodies, as well as proliferative responses of lymphocytes to homologous or heterologous L1 VLPs [7]. Similar results were obtained in a double-blind randomized, placebo-controlled dose-escalation trial to evaluate the safety and immunogenicity of HPV Type 16 L1 VLPs in 72 healthy adults. Interestingly, HPV VLPs were highly immunogenic even without adjuvant and most of the recipients achieved serum antibody titers that were approximately 40-fold higher than the levels observed in natural infection [8].

It should be noticed that in these studies, VLPs were injected intramuscularly but in a Phase I study, recombinant Norwalk VLPs given orally to 24 adult volunteers were also well-tolerated and immunogenic [9].

Development of virus-like particles as delivery systems to induce protective immunity against heterologous antigens

In order to generate immunogens containing well-defined B- and T-cell epitopes, considerable efforts have been put in to develop particulate antigen presentation and carrier systems. Based on the intrinsic potential of native VLPs to induce immune responses, chimeric VLPs carrying heterologous epitopes have been engineered. The development of hybrid VLPs has proven that nonreplicating vectors represent an efficient and safe strategy for eliciting immune responses. In addition, these particles can be produced in large quantities and are easily purified. Here, we will focus on four powerful antigen delivery systems based on recombinant VLPs: HBsAg, Ty-VLPs, PPV-VLPs and PV-VLPs.

HBsAg particles

The hepatitis B virus (HBV) small envelope protein (HBsAg-S) has the ability to self-assemble into VLPs without any contribution of nucleocapsid [10]. Native 22 nm particles, containing about 100 HBsAg-S molecules, are surprisingly effective in priming cellular and humoral responses, even in the absence of adjuvant and these particles are used successfully worldwide for hepatitis B vaccination.

The ability of HBsAg particles to also deliver heterologous antigens when genetically inserted into the external hydrophilic loop was demonstrated 15 years ago. Indeed, hybrid HBsAg particles expressing a poliovirus neutralization epitope were recognized by poliovirus-specific antibodies and induced neutralizing antibodies against the poliovirus [11]. Due to their ability to accept peptidic insertions, HBsAg particles were used as a carrier molecule for selected epitopes of HIV-1 and HCV viruses. Very interestingly, these recombinant particles were proven to be immunogenic in rodents and to some extent, in primates [11–13].

HBsAg were also used to deliver a parasite antigen, the circumsporozoite surface (CS) protein of *P. falciparum*, in an attempt to design an efficient prophylactic vaccine against malaria. After initial analysis in experimental models [14], the response to CS HBsAg was evaluated in humans [15,16]. Lalvani *et al.*, demonstrated that this RTS,S vaccine induced both specific proliferative responses, characterized by a Th1 profile and strong antibody responses in healthy volunteers [17], associated with protection against a challenge with the parasite [18]. More recently, the efficacy of recombinant CS/HBsAg particles was evaluated in Gambia in a randomized trial in 397 semi-immune adult men exposed to natural infection. The results were very encouraging since this pre-erythrocytic RTS, S/AS02 malaria vaccine was safe, immunogenic and induced a partial protection against natural *P. falciparum* infection [15].

Ty virus-like particles

The yeast retrotransposon, Ty, contains in its transcriptional unit, two overlapping open-reading frames, TYA and TYB, analogous to retroviral Gag and Pol genes. TYA encodes a protein, p1, that has the ability to self-assemble into virus-like particles (Ty-VLPs). To produce hybrid Ty-VLPs, genes encoding the protein of interest were inserted at the 3′ end of the truncated TYA gene, resulting in the generation of a p1-fusion protein. The versatility of this antigen delivery system is exceptional since Ty-VLPs can accommodate a wide range of polypeptidic sequences from 1 to 43 kDa, without disrupting the integrity of the particle [19]. These recombinant particles have been shown to be immunogenic, eliciting antibodies, T-helper and CTL responses against the inserted antigens. Due to their immunological properties, hybrid particles containing heterologous epitopes from the HIV-1 core p24 and envelope gp120 (V3 loop) proteins were developed in an attempt to develop a therapeutic vaccine against AIDS. When used to immunize animals, including primates, hybrid p24 Ty-VLPs and V3 Ty-VLPs induced HIV-specific neutralizing antibodies and proliferative T-cell responses [19–21]. In mice, hybrid V3 Ty-VLPs induced V3-specific CTL in the absence of adjuvant after one single *im.* injection [22]. In addition, recombinant Ty-VLPs are also potent systems for inducing CTL to a variety of other viral epitopes, including influenza virus, sendai virus and vesicular stomatitis virus nucleoproteins expressed as polyepitopes [23]. The intact particulate structure of hybrid Ty-VLPs was shown to be essential for CTL induction [24].

Porcine parvovirus VLP

The major structural protein VP2 of porcine (PPV) and canine parvovirus (CPV) has the property to self-assemble into 25 nm pseudo viral particles when expressed in insect cells using the baculovirus expression system. The C-terminus integrity of the VP2 was initially shown to be essential for preserving the capsid structure whereas insertion of heterologous epitopes with appropriate flanking residues at the VP2 N-terminus did not alter the particle formation [25]. Insertion of T-cell epitopes at the N-terminus of VP2 provided a very efficient strategy to stimulate potent Th and CTL responses against foreign antigens without additional adjuvant in mice [26,27]. Indeed, a single ip. immunization with VLPs formed by a VP2 hybrid protein carrying a $CD8^+$ T-cell epitope from the lymphocytic choriomeningitis virus (LCMV) nucleoprotein induced strong CTL responses against both peptide-coated and virus-infected-target cells. Further analysis revealed that PPV-VLPs induced a high frequency of cytotoxic T-lymphocytes as compared to other delivery vehicles, such as microspheres [28]. Indeed, PPV-VLPs stimulated six-times more specific T-cells than microspheres, while carrying 100-times less LCMV peptide and induced long-lasting memory $CD8^+$ T-cells with cytolytic activity which had persisted for at least 9 months [26,28]. Potent CTL responses generated by chimeric LCMV-PPV-VLPs were shown to be associated with viral protection. Indeed, recombinant PPV-VLPs expressing the NP118-132 epitope from LCMV induced a full protection against a lethal challenge with LCMV in adult mice [26]. More recently, this observation was extended to neonate mice, suggesting that chimeric PPV-VLPs represent attractive prophylactic candidates to induce protective immunity in early life [29].

The N-terminus of VP2 protein was also shown to accommodate MHC Class II epitopes derived from HBsAg and poliovirus. The presentation of these epitopes by MHC Class II molecules was estimated to be 100-fold more efficient when expressed by PPV-VLPs as compared with free peptide. A single s.c. immunization with these recombinant PPV-VLPs was sufficient to induce a strong $CD4^+$ T-cell response against the inserted epitope, characterized by a Th1-like cytokine profile [27].

While the N-terminus of VP2 is probably the best site for genetically inserted T epitopes, it is completely hidden inside the capsid, being inaccessible for antibodies. Analysis of the humoral response to chimeric PPV-VLPs containing B-cell epitopes at different positions within accessible loops revealed that the insertion sites caused dramatic changes in the antibody response. Interestingly, one insertion site within loop 2 was suitable for the induction of high antibody response [30].

Papillomavirus VLP

Papillomavirus VLPs (PV-VLPs) are nonenveloped icosahedral structures, consisting of a regular array of 72 pentameric capsomeres composed of the major capsid protein, L1. They have the ability to induce a strong B-cell response at a low dose without adjuvant and are the basis for papillomavirus vaccines now being in clinical trials. Therefore, it was important to determine whether PV-VLPs could deliver cytotoxic T- and B-cell epitopes genetically inserted into an immunodominant region of the L1 protein. Peng *et al.* have shown that a heterologous CTL epitope added to the C-terminus of the bovine PV L1 protein could be delivered to the MHC Class I pathway [31]. Given that up to 60 amino acids could be fused to the C-terminus of the truncated L1 protein without disrupting its ability to form VLPs, multiple CTL and B-cell epitopes were introduced into PV-VLPs as a polyepitope. CTL responses were induced against all epitopes whereas a weak specific antibody response was also measured in mice immunized with polyepitope PV-VLPs in the absence of adjuvant [32]. In addition, PV-VLPs could deliver tumor-associated antigen-like P1A and trigger an antitumor CTL response associated with a therapeutic antitumor response [33]. More recently, PV-VLPs were used to vehicle heterologous autoantigens in order to stimulate specific autoimmune humoral responses in the host against potentially pathogenic molecules [34,35]. Indeed, a peptide representing an extracellular loop of the mouse chemokine receptor CCR5 was incorporated into an immunodominant site of the bovine PV L1 protein. The chimeric VLPs injected to mice induced autoantibodies that inhibited binding of CCR5 to its ligand RANTES and blocked HIV-1 infection of an indicator cell [34]. While the genetic insertion of antigenic sequences into L1 gene represents the most widely used technique to express heterologous antigens, the ability of chimera to self-assemble into functional PV-VLPs was highly unpredictable. To circumvent this problem, Chackerian *et al.* have developed a more flexible and reliable method for displaying a given peptide on VLPs by linking streptavidine-peptide fusion protein to native VLPs [35]. Obviously this approach represents a critical advance in the production of VLPs carrying foreign sequences.

Recombinant VLPs induce systemic & mucosal protective immunity

The humoral response can be an essential component of the protective immunity against infectious agents. The VLPs mentioned above were all shown to induce high-titer neutralizing antibodies in rodents [11,19,36,37]. Interestingly, immunization of primates with HBsAg chimera carrying HIV-1 major envelope neutralizing determinants stimulated proliferative T-cell response and in some instances, neutralizing antibodies and antibody-dependent cellular cytotoxicity [11]. While a correlation was initially established between the induction of high-titer antibodies and the protection in chimpanzees, it remained to be determined to which extend these responses contributed efficiently to the protection against HIV. Very recently, different studies demonstrated the efficiency of VLPs carrying HIV-1 epitopes in the induction of both cellular and humoral responses in nonhuman primates [38,39]. Thus, designing VLPs with multiple T and B epitopes from HIV-1 could represent a promising approach in the vaccination against AIDS.

While the induction of systemic immunity is essential to eradicate microorganisms that already invaded the body, it is of great importance to first elicit an immune response at the mucosal surface to block, or at least limit the entry of pathogens. It became clear that the route of administration of chimeric

VLPs is determinant in inducing mucosal immune responses [36]. Of main interest, mucosal delivery of recombinant VLPs in mice and primates induced both mucosal and systemic immunity and in addition, triggered cellular immunity to inserted T-cell epitopes. It has been demonstrated that intranasal, but not oral delivery of PPV-VLPs in mice, in the absence of adjuvant, elicited strong PPV-specific IgA and IgG antibodies at mucosal sites, as well as serum neutralizing antibodies [39]. Similarly, immunization with chimeric E7:PPV-VLPs *via* the intranasal (in.) route, elicited systemic and mucosal antibody responses, characterized by higher local IgA and neutralizing IgG response in lung [41]. Moreover, systemic-specific CTL responses were also induced in mice by recombinant PPV-VLPs carrying the LCMV epitope administered in. [39]. In primates, sequential mucosal immunizations with chimeric VLPs induced very potent mucosal and systemic immune responses. Vaginal followed by oral, or oral followed by rectal, administration of recombinant SIV gag p27: Ty-VLPs induced specific secretory IgA and IgG locally, as well as specific CD4+ T-cell proliferation in the draining lymphoid organs. Additional systemic cellular and antibody responses were also measured in the spleen and blood of immune animals [42,43].

These observations highlight the potential of VLPs as a powerful delivery system to trigger immune responses when administered either systemically or at mucosal sites. The protection induced in volunteers against *P. falciparum* natural infection by recombinant HBsAg carrying the CS protein is a very strong argument that this strategy could be fully successful in humans.

Mechanisms of CR activation by VLPs

In spite of abundant experimental data showing that VLPs are very efficient inductors of immune responses, few efforts have been devoted to unravel the mechanisms responsible for such a powerful capacity. However, quite recently, several studies have identified some of the VLPs properties, that could be responsible for their immune activities.

Exogenous antigens are cross-presented to CD8+ T-cells by dendritic cells

The induction of specific cytotoxic T-lymphocytes responses usually requires the processing of antigens inside the cytosol of antigen-presenting cells (APCs), followed by the association of derived peptides to MHC Class I molecules. Both macrophages [44] and DCs [45] have been reported to act as APCs but only DCs are able to stimulate naive CD8+ T-cells [46]. Indeed, APCs have to be activated in order to deliver to T-lymphocytes costimulatory signals, which will promote either activation or tolerance of these cells.

Since exogenous antigens, such as noninfectious, nonreplicative VLPs, do not reach the cytosol of APCs, in theory, they cannot be presented by MHC Class I molecules. However, recently it has been shown that exogenous antigens can induce MHC I restricted CTL responses by an alternative pathway, called cross priming, by M Bevan [47,48]. In fact, several alternative pathways have been recently described which are specialized for the processing of exogenous antigens and their presentation by MHC I [49–51].

Initially, the concept of cross priming referred to the transfer of cell-associated antigens from cells that expressed or carried them to APCs and their subsequent presentation by MHC I. It has recently been extended to the presentation of exogenous antigens to CD8+ T-cells by macrophages (Mϕ) and DCs [52]. Two mechanisms of cross priming have been described involving either the transfer of antigen from endosomes to cytosol or its processing inside the endosomes [51]. The first route is used mostly by DCs whereas the second one is specific to macrophages [53].

HBsAg particles

Both DCs and Mϕ can prime $CD8^+$ T-cells *in vivo* to HBsAg particles [54]. However, HBsAg particles are presented to $CD8^+$ T-lymphocytes by several cell types, including mastocytoma cells, fibroblasts, DC lines, Mϕ lines and B- and T-lymphocytes, indicating that a general mechanism of uptake and processing is common to these cells [55].

Since the internalization of HBsAg particles is cytochalasin B-resistant (an inhibitor of actin polymerization), fluid-phase noniclathrin-mediated endocytosis or macropinocytosis should be responsible for the uptake of this 20–30 nm particle. The analysis of presentation to T-cells of HBsAg particles using Mϕ and DCs as APCs demonstrated that this processing is brefeldin A (BFA)-resistant and TAP-independent, showing that this uptake requires metabolically active cells and acid proteolysis within an endosomal or lysosomal compartment [56,57]. Therefore, HBsAg particles are processed inside endocytic vesicles of Mϕ and do not reach the cytosol of APCs. In contrast, the processing of heat-denatured 1–2 μm HBsAg aggregates is cytochalasin B-sensitive and involves the regurgitation of antigenic peptides [55].

'Empty' Class I molecules expressed at APCs cell surface could play a role in HBsAg particles presentation. Indeed, after internalization from the cell surface of APCs, empty L^d molecules are transported to endosomal compartments, where they are loaded with antigenic peptides derived from endocytosed HBsAg particles. These very unstable peptide-L^d complexes are stabilized by their association with β2 microglobulin (β2M) and recycle back to the cell surface of APCs to present the HBsAg derived-epitopes to CD8+ T-cells [58]. Antigen presentation assays performed in serum-free medium revealed that processing of HBsAg for MHC I-restricted presentation depends on exogenous β2M [59].

HBcAg particles

HBcAg particles are 30 nm particles formed by the HBV core antigen. Initially, it was showed that the injection of HBcAg particles to mice without adjuvant efficiently primes antibody responses but does not induce CTL responses, [60]. However, two recent studies reached opposite conclusions [61,62].

DCs – and with a lower efficacy – Mϕ, pulsed *in vitro* or *in vivo* with HBcAg particles carrying heterologous epitopes, can present these epitopes to CD8+ T-cells, whereas B- or T-lymphocytes cannot process these particles.

Both DC subpopulations, $CD8\alpha^+$ and $CD8\alpha^-$ DCs, can present HBcAg particles, although the $CD8\alpha^+$ DCs are more efficient. *In vitro* as well as *in vivo*, the presentation of HBcAg particles, by DCs is partially TAP-dependent, whereas their presentation by Mϕ is fully TAP-dependent, showing that these APCs use different pathways for HBcAg processing [61]. Intradermal injection of mice with HBcAg induced the expression of DCs costimulatory surface molecules (such as CD40, CD80 and CD86) on DCs. However, despite the induction of DC maturation these hybrid particles were unable to induce CTL responses in the absence of adjuvant.

Papillomavirus-VLPs

Using chimeric human papilloma virus type-16 VLPs (HPV16 VLPs) formed by L1 and L2 proteins from the viral capsid, Kast *et al.* showed that PV-VLPs bind *in vitro* to human DCs and are taken up by these cells [63]. In addition, HPV16 VLPs induced the expression of costimulatory molecules (such as MHC I and II as well as CD80, CD83 and CD86 molecules) and secretion of IL-12p70 by DCs. The capacity of these VLPs to induce DCs maturation could explain the ability of PV-VLPs-based vaccines to induce potent T-cell responses in the absence of adjuvant.

The group of JT Schiller who compared several human and bovine PV-VLPs, demonstrated that these various particles are effectively bound and rapidly internalized by murine bone marrow DCs [64] and trigger the expression of costimulatory molecules by these cells. Strikingly, polyomavirus VLPs – which are nonenveloped icosahedral particles structurally similar to PV – bound to the DC surface and were internalized, but failed to induce maturation [64].

The *in vitro* uptake of HPV16 VLPs is dependent of an intact actin cytoskeleton, which could suggest that is mediated by an active uptake mechanism [63], such as endocytosis. Several molecules have been proposed as putative receptors for HPV-VLPs. Indeed, CD16 has been proposed as a receptor for HPV-16 VLPs [65], α_6 integrin subunit (CD49f) for HPV-6b VLPs [66] and heparin and cell-surface glycosaminoglycans on human keratinocytes for HPV-11 VLPs [67].

PPV-VLPs

We recently demonstrated that only PPV-VLPs pulsed DCs can efficiently induce a CTL response *in vivo* to heterologous epitopes carried by these recombinants PPV-VLPs. Conversely, other putative APCs, such as Mϕ and B-cells pulsed with these particles are unable to elicit a CTL response. PPV-VLPs are mainly and very efficiently captured by DCs, although Mϕ and $B220^+$ cells can also capture PPV-VLPs at very low intensity [68]. The uptake of PPV-VLPs is dimethylamiloride-sensitive; thus showing that macropinocytosis is involved in this process. The processing of PPV-VLPs by splenic DCs is fully abrogated in $TAP1^{-/-}$ mice [68]. Furthermore, PPV-VLPs processing depends upon the proteasome complex and is inhibited by LLnL and lactacystine. However, PPV-VLPs processing also requires vacuolar acidification and some lysosomal proteases (in particular pepstatine), indicating that the first steps of PPV-VLPs processing could occur in vesicular compartments. In contrast to HBsAg VLPs, recycling processes are not involved in this processing and *de novo* protein synthesis is necessary for their presentation (unpublished results).

Injection of PPV-VLPs to naïve mice induces the increase of expression of DC MHC class I and II molecules, as well as the expression of costimulatory molecules, such as CD40, CD80 and CD86 [68]. These features associated to the fact that PPV-VLPs processing occurs in the cytosol of DCs, a pathway that has been shown to be much more efficient than the TAP-independent pathways [69], can explain the particular abilities of these recombinant hybrid VLPs to elicit CTL responses to the inserted antigens.

In conclusion, the VLPs described above take advantage of different pathways of processing – showing that MHC I processing is not as rigid as assessed several years ago. However, our knowledge of the mechanisms used by the immune system to recognize and respond to VLPs is still rather limited. More exhaustive studies will be necessary in order to reveal the features of the VLPs processing pathways and to take advantage of them for the design of optimized vaccines.

Summary & conclusions

Usually, purified proteins are not immunogenic and require strong adjuvant in order to induce immune responses. Furthermore, such exogenous antigens cannot reach the cytosol of APCs and are therefore unable to stimulate CTL responses. These properties have limited the development of antiviral vaccines based on purified antigens, even though such vaccines are safer than live attenuated vaccines, in particular in immunodeficient individuals. The high immunogenicity of VLPs, injected without any adjuvant, is therefore a highly surprising finding that could dramatically change our vision of the development of antiviral vaccines. The fact that VLPs exploit alternative strategies to reach the cytosolic pathway for MHC I presentation is one clue to understand their unexpected capacity to induce CTL responses. The second finding, explaining this high immunogenicity is that several of these VLPs have been shown to induce the maturation of DCs. This property could be a great advantage over live attenuated vaccines. Many viruses have developed escape mechanisms leading, in many cases, to the paralysis of APCs whereas VLPs only retained signals capable to stimulate the innate immunity. Elucidation of the various pathways leading to the induction of cellular immunity by VLPs would certainly bring important information for the development of a new generation of VLPs antiviral vaccines. In the meantime, the clinical development of a VLP-based therapeutic antiPV vaccine, as well as antimalaria RTS,S vaccine, will, without any doubt, pave the way for the future of this strategy of vaccination.

Expert opinion

VLPs have unique properties that only start to be recognized. Presumably due to their size and structure, their capture by APCs is highly efficient and leads to delivery to cell

compartment, normally forbidden to purified proteins. Moreover, at least some VLPs, such as PPV-VLPs, are endowed of intrinsic adjuvanticity avoiding the requirement for adjuvants capable to stimulate strong Thelper and CTL responses. Whether these findings obtained with a limited number of particles will be extended to more VLPs is still difficult to predict. However, considering the high efficacy of the HBsAg anti-HBV vaccine, one could be astonished that VLPs have not been used more often for the development of antiviral vaccines. One obvious explanation is the much lower costs required for producing live attenuated vaccines as compared with the sophisticated, and high cost, technologies required for the production and purification of VLPs. This explains why such a strategy has been developed for HBV, which cannot be grown *in vitro*. Another potential limitation of this delivery system could be related to the effect of pre-existing anti-VLPs immunity on the responses induced against epitopes or antigens delivered by the same recombinant VLPs. Indeed, it was recently shown that anti-HPV neutralizing antibodies inhibited the antitumor response induced by chimeric HPV-VLPs [76]. It should, however, be mentioned that in the case of PPV-VLPs, priming against the VLPs only transiently diminished the cellular responses induced by recombinant PPV-VLPs [36]. Moreover, this limitation is in fact observed for most vectors and could be overcome by prime-boost protocols of immunization based on different delivery systems. Therefore, in conclusion, the increasing demonstration of the efficacy of VLPs-based vaccines in preclinical models as well as the results of first clinical trials would support the potential development of VLPs for therapeutic anticancer vaccines.

Five-year view

The key issue for consideration concerns the possibility that these new strategies, developed in many cases in academic laboratories, could reach the market in a relatively short time. In that respect, the results obtained with the antimalaria RTS,S vaccine will be of major importance. Indeed, even if the observed protection persisted only for few months, it is, however, the first time that such a level of protection is demonstrated in malaria. If protection could be confirmed and improved — and that certainly will be done in the next 5 years — this success will open the way for using similar hybrid VLPs for other vaccines.

One could also expect important developments in the field of anticancer vaccines. Indeed, this field is particularly active and often based on strategies difficult to use in routine, such as the injection of DCs loaded with peptides or tumor lysates. The fact that VLPs are targeted to DCs and reach very efficiently the MHC I pathway could have important implication for the development of anticancer vaccines based on such VLPs carrying tumor antigens or epitopes. Alternatively, in the next 5 years, the efficacy of the anti-PV VLPs vaccines in cancer patients will be certainly known, opening the way to a real evaluation of such strategies.

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Key issues

- Nonreplicative virus-like particles (VLPs) are highly immunogenic and induce strong CTL responses.
- Although noninfectious, VLPs are efficiently delivered to MHC I molecules and induce CTL responses. Different VLPs could exploit different processing pathways.
- VLPs induce dendritic cell maturation leading to high immunogenicity.
- An antimalaria candidate vaccine based on recombinant hybrid HBsAg particles is safe and induce protection against natural infection.
- Anticervical cancer vaccines based on papillomavirus VLPs are well-tolerated and induce immune responses in healthy volunteers.
- VLPs represent a new and promising strategy to deliver epitopes or antigens to the immune system for prophylactic and therapeutic vaccines.

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